frontiers in
ENVIRONMENTAL SCIENCE

Wastewater Management

Biological reduction of iron to the elemental state from ochre deposits of Skelton Beck in Northeast England

Pattanathu K S M Rahman and Suvechhya Bastola

Journal Name:	Frontiers in Environmental Science
ISSN:	2296-665X
Article type:	Original Research Article
Received on:	02 Apr 2014
Accepted on:	01 Jun 2014
Provisional PDF published on:	01 Jun 2014
www.frontiersin.org:	www.frontiersin.org
Citation:	Rahman PK and Bastola S(2014) Biological reduction of iron to the elemental state from ochre deposits of Skelton Beck in Northeast England. *Front. Environ. Sci.* 2:22. doi:10.3389/fenvs.2014.00022
/Journal/Abstract.aspx?s=1483& name=wastewater%20management& ART_DOI=10.3389/fenvs.2014.00022:	/Journal/Abstract.aspx?s=1483& name=wastewater%20management&ART_DOI=10.3389 /fenvs.2014.00022
	(If clicking on the link doesn't work, try copying and pasting it into your browser.)

Biological reduction of iron to the elemental state from ochre deposits of Skelton Beck in Northeast England

Pattanathu K.S.M. Rahman* and Suvechhya Bastola

Technology Futures Institute, School of Science and Engineering, Teesside University, Middlesbrough –TS1 3BA, Tees Valley, United Kingdom.

Tel: +44-1642-384669; E-mail: p.rahman@tees.ac.uk

*** Author for correspondence**

ABSTRACT

Ochre, consequence of acid mine drainage, is iron oxides-rich soil pigments that can be found in the water drainage from historic base metal and coal mines. The anaerobic strains of *Geobacter sulfurreducens* and *Shewanella denitrificans* were used for the microbial reduction of iron from samples of ochre collected from Skelton Beck (Saltburn Orange River, NZ 66738 21588) in Northeast England. The aim of the research was to determine the ability of the two anaerobic bacteria to reduce the iron present in ochre and to determine the rate of the reduction process. The physico-chemical changes in the ochre sample after the microbial reduction process were observed by the production of zero-valent iron which was later confirmed by the detection of elemental Fe in XRD spectrum. The XRF results revealed that 69.16% and 84.82% of iron oxide can be reduced using *G. sulfurreducens* and *S. denitrificans* respectively after 8 days of incubation. These results could provide the basis for the development of a biohydrometallurgical process for the production of elemental iron from ochre sediments.

Key words: Iron, Ochre, Mine water treatment, *Geobacter sulfurreducens, Shewanella denitrificans*

INTRODUCTION

Water seepage from old ironstone mine workings into Skelton Beck is caused by rising ground-water levels from the old Longacre Pit in Skelton, which is choked with iron ochre. The iron deposits have coloured the beck red and threaten wildlife. Government agencies worldwide often face challenges towards remediation of water contaminated by historical mining activities (Mayes et al. 2009).

Acid Mine Drainage (AMD) is one of the most challenging environmental problems faced today due to historic mine activities. Looking at the mining history it has been stated that a total of about 1,150 million tons of heavy metals have been mined since the Stone Age (Sheoran and Sheoran, 2006; Simpson et al. 2014). The typical characteristic of AMD is the significantly low pH and high concentration of heavy metals (Engleman and McDiffett, 1996). This problem results from the microbial oxidation of iron pyrite in the presence of water and air generating acidic leachates containing toxic metal ions and sulphates (Mohan and Chander, 2006).

The oxidation of iron disulfides (pyrite) occurs in the beginning, this oxidation reaction may release ferrous iron, sulfate and H^+ in the aqueous solution (Reaction 1). Ferrous iron is simultaneously oxidized to the ferric form (Reaction 2), which is hydrolysed and ferric hydroxide is generated.

$$\mathbf{2FeS_2}\ (\text{Pyrite}) + \mathbf{7O_2} + \mathbf{2H_2O} \longrightarrow \mathbf{2Fe^{2+}}\ (\text{ferrous iron}) + \mathbf{4SO_4^{2}}\ (\text{Sulphate}) + \mathbf{4H^+}\ (\text{acid}) \quad (1)$$

$$4Fe^{2+} + O_2 + 4H^+ \longrightarrow 4Fe^{3+} + 2H_2O \quad (2)$$

Komnitsas and Pooley (1990) reported acceleration of pyrite oxidation by bacterially generated ferric iron. Other microorganisms like *Thiobacillus ferrooxidans* derive energy from the oxidation of ferrous to ferric iron (Cheng et al, 2009).

$$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+ \quad (3)$$

Thus it can be observed that eventually SO_4^{2-} concentration is increased (Reaction 3) and the pH is decreased due to the iron disulphide dissolution. Although the reactions causing oxidation of pyrite and formation of AMD occur in an abiotic environment, lithotrophic microbes can be helpful to accelerate this process (Komnitsas and Pooley, 1990; Mayo et al., 2000; Cheng et al, 2009).

One of the consequences of acid mine drainage is the formation of ochre. It has been reported that the term "ochre" is used for materials having iron or iron–rich ore minerals in the range of 3 to 30% which are found in the water drainage from coal and other mines (Popelka-Filcoff, 2008). The main chromophores responsible for the red and yellow pigments are hematite (α-Fe_2O_3) and goethite (FeO(OH) respectively (Gill et al., 2007). At high Fe(III) and sulphate concentrations and low pH jarosite, capable of adsorbing several other heavy metals present in AMD, is formed (Komnitsas et al., 1995).

The objective of the study is to develop a sustainable procedure to extract elemental iron from the naturally prevailing and hazardous ochre. The water in Skelton Beck is regarded as harmful to human and aquatic lives and had been a matter of concern since 1866, the evidence of which can be found in the papers published in "The Lancet" (Rapp et al., 1866).

This is the first attempt of its kind to develop a microbial treatment process for the ochre in its natural environment, which will make the procedure more promising and reliable.

MATERIALS AND METHODS

PREPARATION OF OCHRE SAMPLES

The ochre sample was collected from Skelton Beck river bed, receiving acid mine discharge. Prior to the experiment, the ochre sample was pre-dried in an oven at 40^{o}C for 12 h. The dried sample was slowly grinded to obtain fine powder (<10 µm), which was then sieved manually with the help of sieve with apertures of 500 µm and stored in refrigerator for further use. The Scanning electron microscope image of the fresh ochre is presented in Figure 1. Some agglomeration as a result of drying was noticed.

PRE-CULTURE OF THE TWO BACTERIAL STRAINS TO PREPARE BIOMASS FOR INOCULATION

Anaerobic bacterial strains used were *Geobacter sulfurreducens* (DSM 12127) and *Shewanella denitrificans* (DSM 15014). The strains were selected based on their ability to solubilise minerals (Table 1). Sterile nutrient broth (CM0001, Oxoid, UK) was used as medium for *G. sulfurreducens* (*GS*) and *S. denitrificans* (*SD*). It was prepared with de-ionised water for *GS* and filtered sea water for *SD* respectively. Abiotic and biotic controls were maintained for both the cultures. 10 mL of inoculum (48 h growth) was inoculated in 90 mL nutrient broth and complete anaerobic condition was maintained in Drechsler bottle by replacing nitrogen gas, which was confirmed with the help of the risazurine anaerobic indicator (BR0055, Oxoid, UK) and 5% air dried ochre was used in the experiment. Both of the cultures were sub-cultured for 48 h. The bacterial growth was measured using UV visible spectrophotometer (Camspec model: M550) at absorbance of 600 nm.

PREPARATION OF SAMPLES FOR ANALYSIS

The sub-cultured microorganisms were inoculated in nutrient broth with ochre and were incubated for 8 days in anaerobic condition. The culture was monitored at regular time intervals (2 days) to assess the rate of iron synthesis from ochre by both the strains. For the analysis of solid residues, the culture broth with ochre was transferred to centrifuge tubes. The centrifugation was carried out at 10,000 rpm for 10 min at 4^{o}C. Absolute ethanol was added to the pellet thus to sterilise any organisms left and was allowed to dry by placing in the vacuum oven at 64^{o}C for 12 h. When the pellet was completely dried, it was homogenised with the help of mortar and pestle until a fine powder was formed, which was then analysed by X-Ray powder diffraction (XRD), X-Ray fluorescence (XRF) and Scanning electron microscope (SEM).

CHEMICAL ANALYSIS (XRF)

The dried sample was used for the analysis. The XRF analysis was carried out at 30 kV and 150 µA using Eagle III Micro-XRF. Vacuum was maintained inside the XRF for the entire sample analysis. The spectrum was collected after 300 scans of 0.5mm diameter of the sample.

X-RAY DIFFRACTION ANALYSIS

The X-ray powder diffraction was carried out for all the powdered form of the reduced samples using Cu Kα target. For each sample, the scanning time was set for 50 minutes with graphical axis range from minimum of 2θ = 20° to maximum of 70°. The peak intensity and the peak position for all the samples after the X-ray scanning were obtained and thus used for identifying the different forms of iron present in the sample.

SEM-EDX ANALYSIS

The samples were prepared by mounting 10 mg of each reduced sample on aluminium sample holder using double sided tape. These holders were coated with thin film of carbon. Once inside the SEM, random vertical and lateral movements around the microscope stage was carried out to select the appropriate image of the compound present. Then energy dispersive X-ray analysis (EDX) was used to determine the chemical composition of the selected area. The sample was studied by comparing four different spectrums of the area of interest within the sample, to minimise error.

RESULTS AND DISCUSSION

GROWTH STUDY

The bacterial growth was monitored using UV visible spectrophotometer at the absorbance of 600 nm. The optical densities of the inocula were 0.927 and 1.753 for *GS* and for *SD* respectively. These measurements were used to set-up the experiments.

PHYSICO-CHEMICAL OBSERVATION

After obtaining complete anaerobic conditions inside the Drechsler bottles, they were incubated at 30^{o}C in an incubator. As seen in Figure 2, no distinct change in the colour of the samples was observed until the second day of incubation. Then gradual change in the colour of the liquid was observed as the incubation time increased (Figure 3 for *GS* and Figure 4 for *SD*). It can clearly be seen from the image that the change in colour of the experimental samples inoculated with the *SD* is more distinct than the samples inoculated with *GS*. The *SD* shows distinct black colour within the six days of incubation whereas the sample inoculated with *GS* showed only slight change in the colour. Close observation showed that the black colour particle settled to the bottom of the flask and the liquid showed opaque light black

149 colour, however slight shaking of the flask displayed uniform distinct black colour. This
150 change in the colour could be related to the reduction of the iron oxide present in the ochre to
151 elemental iron. Similar observations were reported earlier. For instance Sun et al. (2006)
152 reported that colour of an ochre sample changed to distinct black due to the formation of iron
153 nanoparticles in colloidal form. Furthermore the bottles incubated for more than one week
154 showed a very distinct and dark black colour for both the strains. This change in the colour
155 was maintained throughout the 8 days possibly due to the absence of any reverse reaction.
156
157 The colour of the biotic control before incubation was yellow, identical to the ochre
158 colour, but as incubation time increased, its colour changed gradually to black (Figure 5).
159 This showed that some indigenous microorganisms present in the ochre were able to bring
160 some biotic change in the ochre in the presence of nutrient broth. Acidophilic
161 microorganisms are reported to be found in these types of extreme environment such as the
162 ochre (Johnson, 1998). Moreover, similar change in the colour of sample observed only in
163 biotic controls and no change in the abiotic control (Figure 5) proves that the reduction
164 process is completely a biotic process. Absence of colour changes in the abiotic control could
165 be due to absence of microorganisms and therefore reduction process could not be initiated
166 and it remained unchanged for the entire 8 days of incubation. Studies have shown that iron
167 oxide is present in all types of ochre. But the variation in their colours can be explained by
168 their composition. Table 2 shows a clear relation between colour of ochre and the
169 concentration of iron oxides in the sample (Elias et al., 2006).
170
171 **EFFECT ON pH**
172 The pH of supernatant in the treated samples was found to be 7.8 for *GS* and 7.2 for *SD*. The
173 pH of both the strains was noticed to decrease during incubation. A similar effect was

174 observed by Sun et al., (2006). Decrease in pH values were observed after the reduction of
175 ochre to elemental iron, due to the generation of protons. This reduction can be described by
176 the following reaction, illustrating the generation of acidity:
177
178 $4FeOOH + CH_3CH(OH)COO^- \rightarrow 4Fe + 3HCO^{3-} + 2H_2O + 2H^+$ (4)
179
180 **XRD PATTERN FOR THE TREATED SAMPLES**
181 The pattern of peaks of all the samples incubated with *GS* were similar to some extent but
182 differed in the number of phases for any particular compound. For the sample inoculated with
183 *GS* (Figure 6a & b) the XRD spectrum indicates some degree of amorphicity in the pattern
184 and also shows the presence of Geothite (iron oxyhydroxide - FeO(OH). The phase of the
185 goethite found was the hydrated goethite. Previous studies have demonstrated that at certain
186 conditions amorphous iron(III) hydroxide are transformed to crystalline iron oxides and
187 oxyhydroxides, such as α-Fe_2O_3 and α-FeOOH (Krehula and Music, 2008).
188 From the XRD spectra presented in Figures 6 and 7, it can be observed that all the samples
189 contain goethite (α-FeOOH) as major iron phase and elemental iron, Fe0, as a secondary
190 crystalline phase. The variation in the intensity of peaks can be related to the total incubation
191 period of the samples, and the type of the microbial strain inoculated. A distinct peak with
192 very high intensity was observed in all the samples inoculated with *SD*, but it was not present
193 in the XRD pattern of the samples inoculated with *GS*. This distinct peak was observed at
194 about $2\theta = 31.90°$, which does not fall under any type of iron oxides, but it can be attributed
195 to the mineral siderite, $FeCO_3$, which is a usual bioreduction product of iron hydroxides
196 (Fredrikson et al., 1998). The patterns of peaks for all the samples inoculated with *SD* are
197 found to be rather identical to each other, though they differ in intensity. The sample

198 inoculated with *SD* (Figure 7a & b) show distinctly different peaks than those obtained from
199 *GS*.
200
201 **XRF ANALYSIS OF TREATED SAMPLES**
202 The XRF analysis was carried out for all the samples including four controls. Quantitative
203 results obtained from the XRF analyses further confirm the presence of relatively high
204 percentage of iron. Along with iron, other elements present were silicon, chloride and
205 calcium. The net weight and atomic percentages are summarized in Table 3. Even though the
206 type of elements present in all the samples was similar, they show some variations in their
207 weight percentage. This variation in their percentage weight was found to be affected by the
208 length of incubation time for each sample. However the percentage of iron gradually
209 increases as the length of incubation time increases. The iron percentage reached from 67%
210 to 69% for *GS* and 65 to 84 for *SD* within the first week of incubation. As the colour of
211 sample inoculated with *SD* changed much faster than the sample inoculated with *GS*, it can be
212 said that *SD* is much more effective and efficient in the reduction process.
213
214 **MINERALOGICAL ANALYSIS USING SCANNING ELECTRON MICROSCOPE (SEM)**
215 The *GS* treated sample was analysed by EDX in SEM. Four different EDX spectra were
216 recorded and the results are presented in Table 4. The main elements were iron (35.4%),
217 oxygen (48.4%), carbon (13.8%), silicon (0.88%) and calcium (1.31%). Four different EDX
218 spectra were also recorded for the sample treated by *SD* (Table 5). In addition to Fe, O, Ca, Si
219 and Cl, the sample also contained Na, Mg and S. The presence of sodium could have been
220 due to the use of sea water in the preparation of nutrient broth for *SD*. However the
221 percentage by weight of sodium, Magnesium and sulfur is almost negligible as they were just
222 1.14%, 0.82% and 0.04% weight respectively. Moreover the sample reduced by *SD* was

found to have higher iron percentage (68.36%) than sample reduced by *GS* (35.36%). Site of observation for the first spectrum of *SD* & *GS* and the elements obtained from the spectrum are presented in Figure 8 and 9.

CONCLUSION

The physio-chemical observation showed that iron oxide can be reduced from ochre by anaerobic bacterial strains namely *GS* and *SD*. The efficiency of the reduction process and the rate of reduction with increasing incubation period were examined. The changes in sample colour from orange to black showed that microbial activities have induced the reduction process. Reduction residues were analysed using XRD, XRF and SEM. Examination of samples by XRD revealed the presence of elemental iron and geothite. XRD data also proved that the peak intensity of geothite, as well as of elemental iron, increased with the increase in incubation period. Moreover, the observation of the change in colour of the samples showed that the reduction process is much faster in case of *SD* in comparison to *GS*.

ACKNOWLEDGEMENT

Authors wish to thank the Dean, School of Science and Engineering for providing facilities, Dr Sayed El-Ghaith and Ms. Aparna J. Padman for their assistance in the laboratory works.

REFERENCES

Cheng, H., Hu, Y., Luo, J., Xu, B., and Zhao, J. (2009). Geochemical processes controlling fate and transport of arsenic in acid mine drainage (AMD) and natural systems. *J. Hazard. Mater.* 165, 13-26. doi:10.1016/j.jhazmat.2008.10.070

248 Elias, M., Chartier, C., Prévot, G., Garay, H., and Vignaud, C. 2006. The colour of ochres
249 explained by their composition. *Mater. Sci. Eng.* 127, 70-80. doi:
250 10.1016/j.mseb.2005.09.061
251
252 Engleman Jr, C. J., and McDiffett, W. F. (1996). Accumulation of aluminum and iron by
253 bryophytes in streams affected by acid-mine drainage. *Environ. Pollut.* 94, 67-74.
254 doi:10.1016/S0269-7491(96)00055-3
255 Fredrikson, J. K., Zachara, J. M., Kennedy, D. W., Dong, H. Onstott, T. C., Hinman, N. W.,
256 Shu-Mei L. (1998). Biogenic iron mineralization accompanying the dissimilatory reduction
257 of hydrous ferric oxide by a groundwater bacterium, *Geochim Cosmochim AC.* 62, 3239-
258 3257. doi: 10.1016/S0016-7037(98)00243-9
259
260 Gil, M., Carvalho, M. L., Seruya, A., Candeias, A. E., Mirão, J., and Queralt, I. (2008).
261 Yellow and red ochre pigments from southern Portugal: Elemental composition and
262 characterization by WDXRF and XRD. *Nuclear Instruments and Methods in Physics*
263 *Research Section A: Accelerators, Spectrometers, Detectors and Associated Equipment* 580,
264 728-731. doi:10.1016/j.nima.2007.05.131
265
266 Johnson, D. B., (1998). Biodiversity and ecology of acidophilic microorganisms. *FEMS*
267 *Microbiol. Ecol.* 27, 307-317. doi:10.1111/j.1574-6941.1998.tb00547.x
268
269 Kato, C., and Nogi, Y. (2001)**.** Correlation between phylogenetic structure and function:
270 examples from deep-sea *Shewanella. FEMS Microbiol. Ecol.* 35, 223–230.
271 doi: 10.1111/j.1574-6941.2001.tb00807.x
272

273 Komnitsas, C., and Pooley, F. D. (1990). Bacterial oxidation of a refractory gold sulphide
274 concentrate from Olympias, Greece, *Miner. Eng.* 3, 295-306, doi: 10.1016/0892-
275 6875(90)90125-U
276
277 Komnitsas, K., Xenidis, A., and Adam, K. (1995). Oxidation of pyrite and arsenopyrite in
278 sulphidic spoils in Lavrion, *Miner. Eng.* 8, 1443-1454. doi: 10.1016/0892-6875(95)00109-3
279
280 Krehula, S., and Music, S., 2008. Influence of cobalt ion in precipitation of goethite in highly
281 alkaline media. *Clay Miner.* 43, 95-105. doi:10.1180/claymin.2008.043.1.07
282
283 Mayes, W. M., Johnston, D., Potter, H. A. B., and Jarvis, A. P. (2009). A national strategy for
284 identification, prioritisation and management of pollution from abandoned non-coal mine
285 sites in England and Wales. I.: Methodology development and initial results. *Sci. Total*
286 *Environ.* 407, 5435-5447. doi:10.1016/j.scitotenv.2009.06.019.
287
288 Mayo, A. L., Petersen, E. C., and Kravits, C. 2000. Chemical evolution of coal mine drainage
289 in a non-acid producing environment, Wasatch Plateau, Utah, USA. *J. Hydrol.* 236, 1-16.
290 doi:10.1016/S0022-1694(00)00277-8
291
292 Mohan, D., and Chander, S. (2006). Removal and recovery of metal ions from acid mine
293 drainage using lignite - A low cost sorbent. *J. Hazard. Mater.* 137, 1545-1553.
294 doi:10.1016/j.jhazmat.2006.04.053
295

296 Popelka-Filcoff, R. S., Miksa, E. J., Robertson, J. D., Glascock, M. D., and Wallace, H.
297 (2008). Elemental analysis and characterization of ochre sources from Southern Arizona. *J.*
298 *Archaeol. Sci.* 35, 752-762. doi:10.1016/j.jas.2007.05.018
299
300 Sheoran, A. S., and Sheoran, V. (2006). Heavy metal removal mechanism of acid mine
301 drainage in wetlands: A critical review. *Miner. Eng.* 19, 105-116. doi:
302 10.1016/j.mineng.2005.08.006
303
304 Rapp, W., Taylor, W., and Cunningham, J. (1866). The sanitary condition of Salt burn. *The*
305 *Lancet,* 88: 505-506
306
307 Simpson, S. L., Vardanega, C. R., Jarolimek, C., Jolley, D. F., Angel, B. M., and Mosley, L.
308 M. (2014). Metal speciation and potential bioavailability changes during discharge and
309 neutralisation of acidic drainage water, *Chemosphere* 103: 172-180,
310 doi:10.1016/j.chemosphere.2013.11.059.
311
312 Sun, Y., Li, X., Cao, J., Zhang, W., and Wang, H. P. (2006). Characterization of zero-valent
313 iron nanoparticles. *Adv. Colloid Interface Sci.* 120, 47-56. doi:10.1016/j.cis.2006.03.001
314
315 Uroz, S., Calvaruso, C., Turpault, M., and Frey-Klett, P. (2009). Mineral weathering by
316 bacteria: ecology, actors and mechanisms, *Trends in Microbiol.* 17, 378-387.
317 Doi:10.1016/j.tim.2009.05.004.
318
319
320

Table 1: Ability of *Geobacter* and *Shewenella* species to solubilize minerals (Kato and Nogi, 2001; Uroz et al., 2009)

Microorganism	Type of microorganism	Natural Habitat	Mechanism involved	Origin of microorganism
Geobacter spp	δ-Proteobacteria	Sub-surface areas	Iron [reduction of Fe(III)]	Sediment
Shewenella spp	γ-Proteobacteria	Deep sea water	Smectite	Anoxic sediment
			Iron [reduction of Fe(III)]	
			Calcite, dolomite	

Table 2: Relationship between color of ochre and its composition (Elias et al., 2006)

Ochre	Presence of hematite	Presence of geothite	Variation in α co-ordinate (Redness)
Red	Yes (>80%)	-	24.6 - 43
Yellow	-	Yes	4.4 - 17.3
Orange	Yes	Yes	19.5 – 34.8

362

363

364 **Table 3: Elemental composition of ochre samples treated with *GS* and *SD* and control**
365 **samples, as determined using XRF analysis**

366

Reduced by	Incubation (Days)	Weight (%)			
		Fe	Ca	Cl	Si
G. sulfurreducens	2	67.38	12.73	13.45	6.44
G. sulfurreducens	4	67.52	15.62	10.22	6.33
G. sulfurreducens	6	69.16	13.77	11.35	5.73
G. sulfurreducens	8	69.16	13.77	11.35	5.73
S. denitrificans	2	65.01	12.37	15.63	6.99
S. denitrificans	4	76.52	3.86	6.19	13.44
S. denitrificans	6	84.74	5.65	7.63	1.98
S. denitrificans	8	84.82	5.83	7.43	1.92
Abiotic control (Distilled water)	8	71.73	-	28.27	-
Abiotic control (Sea water)	8	56.51	-	43.49	-
Biotic control (Distilled water)	8	62.82	7.60	29.59	-
Biotic control (Sea water)	8	21.77	6.05	36.57	-

367

368

369

370

371

372

373

374

375

376

377 **Table 4: Results of EDX-SEM analyses for the ochre sample treated with GS**

Spectrum	In stats.	C	Si	Cl	Ca	Fe	O	Total
1	Yes	13.82	1.03	0.23	1.35	35.01	48.56	100.00
2	Yes	14.02	0.90	0.36	1.21	34.72	48.80	100.00
3	Yes	14.41	0.77	0.37	1.23	33.79	49.43	100.00
4	Yes	12.88	0.84	0.19	1.45	37.92	46.71	100.00
Mean		13.78	0.88	0.29	1.31	35.36	48.38	100.00
Std. Dev.		0.65	0.11	0.09	0.11	1.79	1.17	
Max.		14.41	1.03	0.37	1.45	37.92	49.43	
Min.		12.88	0.77	0.19	1.21	33.79	46.71	

378

379

380

381

382

383

384

385

386

387

388

389

390

391

392

393

394 **Table 5: Results of EDX-SEM analyses for the ochre sample treated with SD**

Spectrum	In stats.	Na	Mg	Si	S	Cl	Ca	Fe	O	Total
1	Yes	1.14	0.81	1.54	0.34	2.19	2.00	68.40	23.59	100.00
2	Yes	1.20	0.78	1.40	0.43	2.18	2.02	68.41	23.58	100.00
3	Yes	1.12	0.84	1.44	0.47	2.14	2.06	68.26	23.67	100.00
4	Yes	1.12	0.85	1.48	0.36	2.23	2.02	68.37	23.57	100.00
Mean		1.14	0.82	1.46	0.40	2.18	2.03	68.36	23.60	100.00
Std. deviation		0.04	0.03	0.06	0.06	0.04	0.02	0.07	0.05	
Max.		14.41	1.03	0.37	1.45	37.92	49.43			
Min.		12.88	0.77	0.19	1.21	33.79	46.71			

395

396

397

398

399

400

401

402

403

404

405

406

407

List of Figures

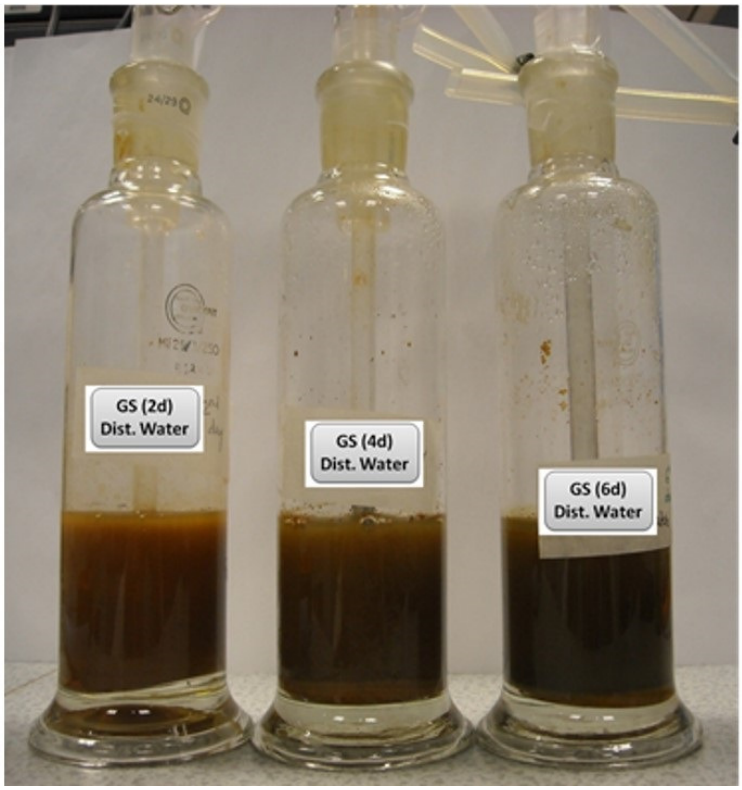
7 (b): after 8 days of incubation

Figure 8. (a) Site of observation for the samples from *GS* treatment (b) Elemental analysis data obtained from spectrum one

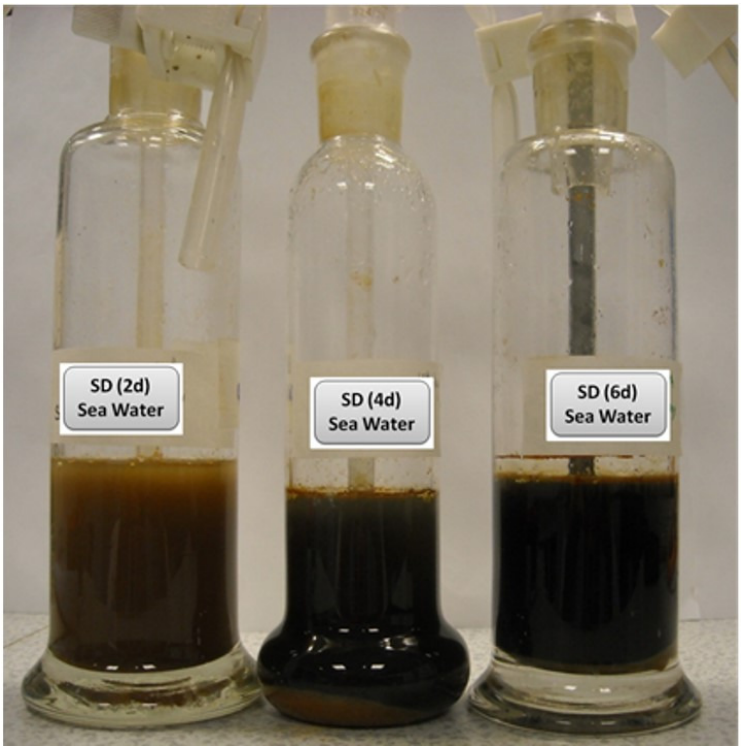
Figure 9. (a) Site of observation for the samples from *SD* treatment (b) Elements obtained from spectrum one

(Fig 1)

(Fig 2)



(Fig 3)



(Fig 4)

514

Biotic control

Abiotic control

515

516

517

518 (Fig 5)

519

520

521

522

523

524

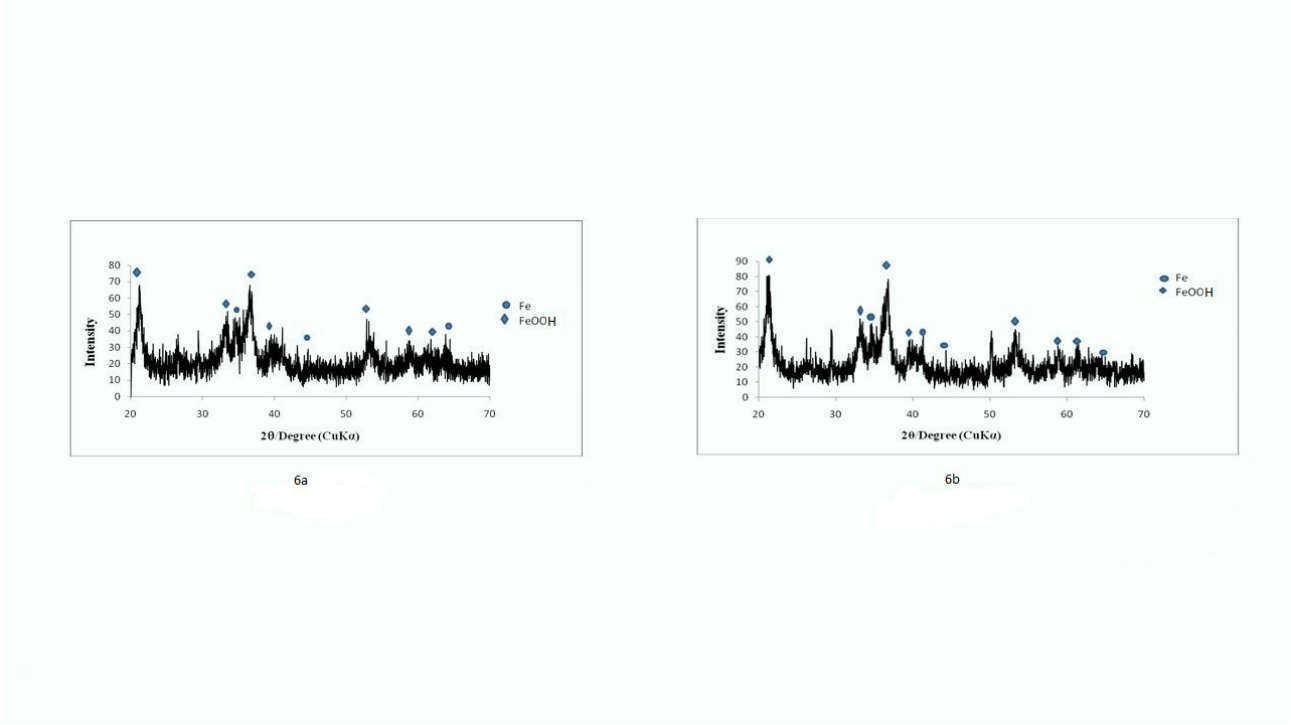
525

526

527

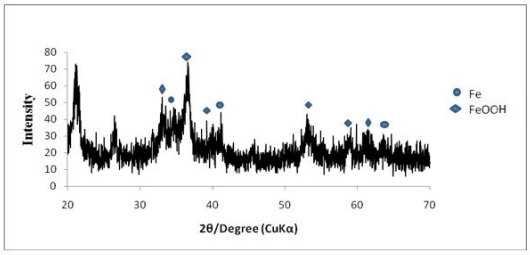
528

529

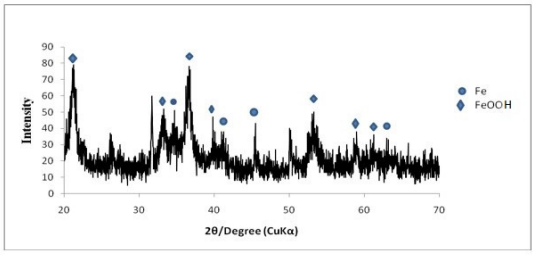

Intensity
2θ/Degree (CuKα)
Fe
FeOOH
6a
Intensity
2θ/Degree (CuKα)
Fe
FeOOH
6b

(Fig 6)

546



7a



7b

547

548

549

550 (Fig 7)

551

552

553

554

555

556

557

558

559

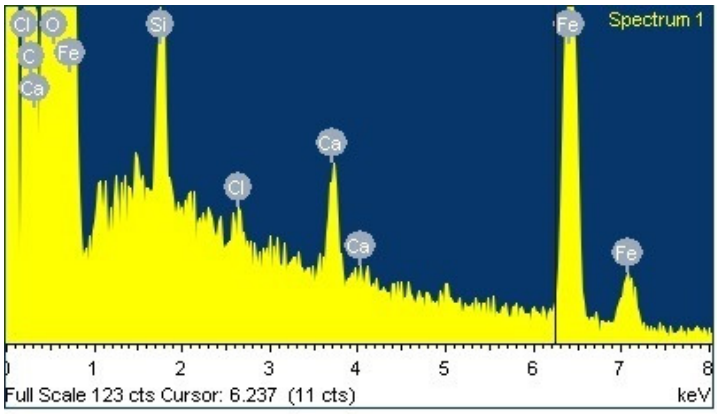
560

561

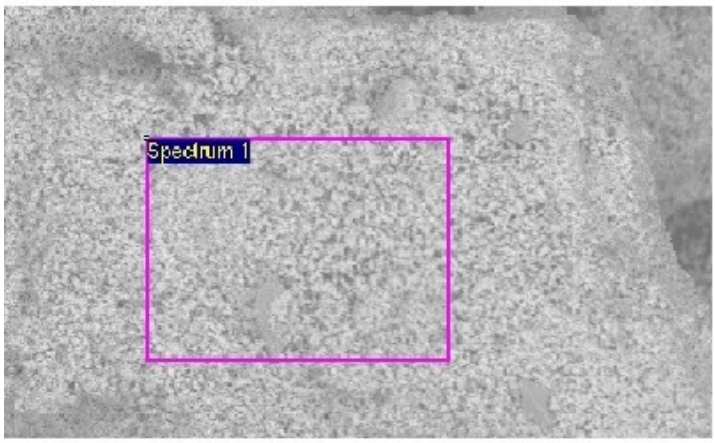
562

563

564



8a



8b

565

566 (Fig 8)

567

568

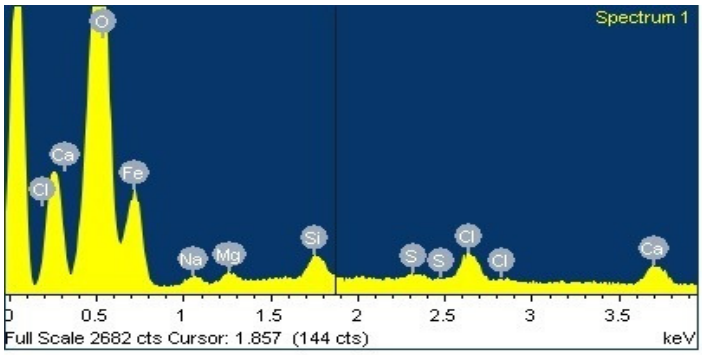
569

570

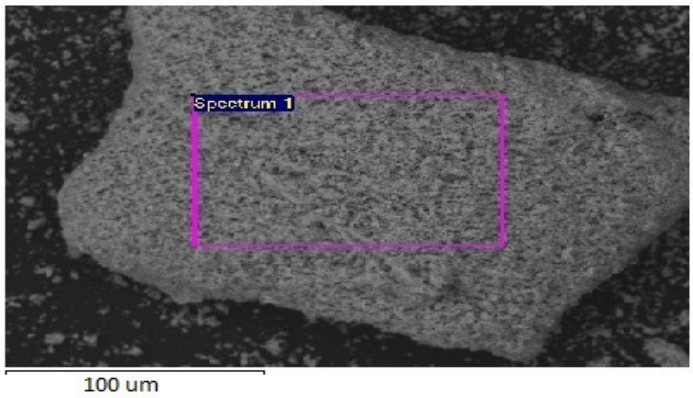
571

572

573



9a



9b

574

575 (Fig 9)